

The University of Chicago

EFFECTS OF SEVERAL
SULFA-COMPOUNDS ON NUCLEAR
AND CELL DIVISION

A DISSERTATION SUBMITTED TO THE FACULTY OF
THE DIVISION OF THE BIOLOGICAL SCIENCES IN
CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF BOTANY

1947

By
THOMAS C. FULLER

Reprinted from
THE BOTANICAL GAZETTE
Vol. 109, No. 2, December, 1947

The University of Chicago

EFFECTS OF SEVERAL
SULFA-COMPOUNDS ON NUCLEAR
AND CELL DIVISION

A DISSERTATION SUBMITTED TO THE FACULTY OF
THE DIVISION OF THE BIOLOGICAL SCIENCES IN
CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF BOTANY
1947

By
THOMAS C. FULLER

Reprinted from
THE BOTANICAL GAZETTE
Vol. 109, No. 2, December, 1947



EFFECTS OF SEVERAL SULFA-COMPOUNDS ON NUCLEAR AND CELL DIVISION¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 593

THOMAS C. FULLER

Introduction

Since the discovery that polyploidy can be produced in plants almost at the will of the investigator through the use of colchicine (2, 3, 4), considerable interest has developed in the possibilities thus opened up, and experiments with other chemicals have been going on. More recently EIGSTI (5) reported that "mitotic irregularities" were induced in the pollen tubes of *Tradescantia occidentalis* when they were treated with dilute concentrations of sulfanilamide (1:10,000). Greater concentrations inhibited the formation of the tube. TRAUB (9) found polyploid ($4n$) and numerous binucleate cells in *Allium* roots after 48 hours of treatment with sulfanilamide (0.5%). PETERS (8) reported that it produced an extreme contraction of the chromosomes of *Allium* at metaphase and inhibited the spindle mechanism. He noted that after reaching the metaphase stage, the chromosomes reverted to an active metabolic condition without completing the mitotic cycle. The reversions occurred both before and after the division of the centromeres, resulting in the production of tetraploid nuclei which were observed in blocked metaphases as early as 8 hours after treatment. He also found that sulfanilamide inhibited the entrance of cells into mitosis and that divisions rarely occurred after 48 hours of treatment. Although less effective than colchicine in

inducing polyploidy in *Allium* (8), sulfanilamide has been more effective in some species of *Vaccinium* (6).

These reports led to a reinvestigation of the effects of sulfanilamide on nuclear and cell divisions, together with the effects of several other sulfa-compounds: viz., sulfadiazine, sulfaguanidine, sulfamerazine, sulfapyridine, and sulfathiazole.

Material and methods

The bases of bulbs of *Allium cepa* L. were placed in tap water in Stender jars in the laboratory for a period of 24-48 hours until roots averaging approximately an inch in length were developed. These roots, while still attached to the bulbs, were treated by immersing them in aqueous solutions of the sulfa-compounds.

Preliminary tests of the effects of sulfanilamide were made in solutions of 0.125, 0.25, and 0.5% concentration by weight in tap water at 20° C. Because the greatest effects occurred in the 0.5% solution, the weaker concentrations were not used in the later experiments. All the other sulfa-compounds were used at 0.1% concentration by weight in tap water because of their low solubility. Even this quantity was slightly above the saturation point of these compounds in water, and a slight excess of solid material was present in each solution.

Treatments were started at 12:30 P.M., and the solutions were changed every 24 hours. Root tips were collected

¹ This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

at intervals up to 96 hours following the start of the treatments. After 48 hours some treated roots were returned to tap water, and collections were made from them 24 and 48 hours later. A few treated roots were also returned to tap water at the end of 96 hours, but, since they showed essentially the same responses as those returned to tap water after 48 hours of treatment, this procedure was not repeated in later experiments. Control bulbs were continued in tap water, which was also changed at 24-hour intervals. Both treated and control root tips were fixed in Navashin's solution, handled according to the tertiary-butyl-alcohol method, and imbedded in paraffin. Sections cut transversely at $20\ \mu$ were stained with Heidenhain's iron-alum hematoxylin with a counterstain of Orange G in clove oil.

Results

The first observable response following the immersion of the roots in the solutions of the sulfa- compounds was the cessation of visible elongation, and it was not renewed during the 96-hour period of treatment. The roots also became progressively discolored with a brownish tinge during treatment.

Approximately half the roots immersed in 0.5% sulfanilamide showed an increase in diameter in the morphological region of elongation within 24 hours. The enlargements reached a maximum size within 48 hours after treatment as a result primarily of an increase in size of the cortical cells. Similar tumors were not formed following treatment with any of the other sulfa- compounds except for one bulb in which an initial swelling was noticed in approximately half the roots after 72 hours of immersion in the sulfa-pyridine solution. These tumors, comparable to those from the sulfanilamide

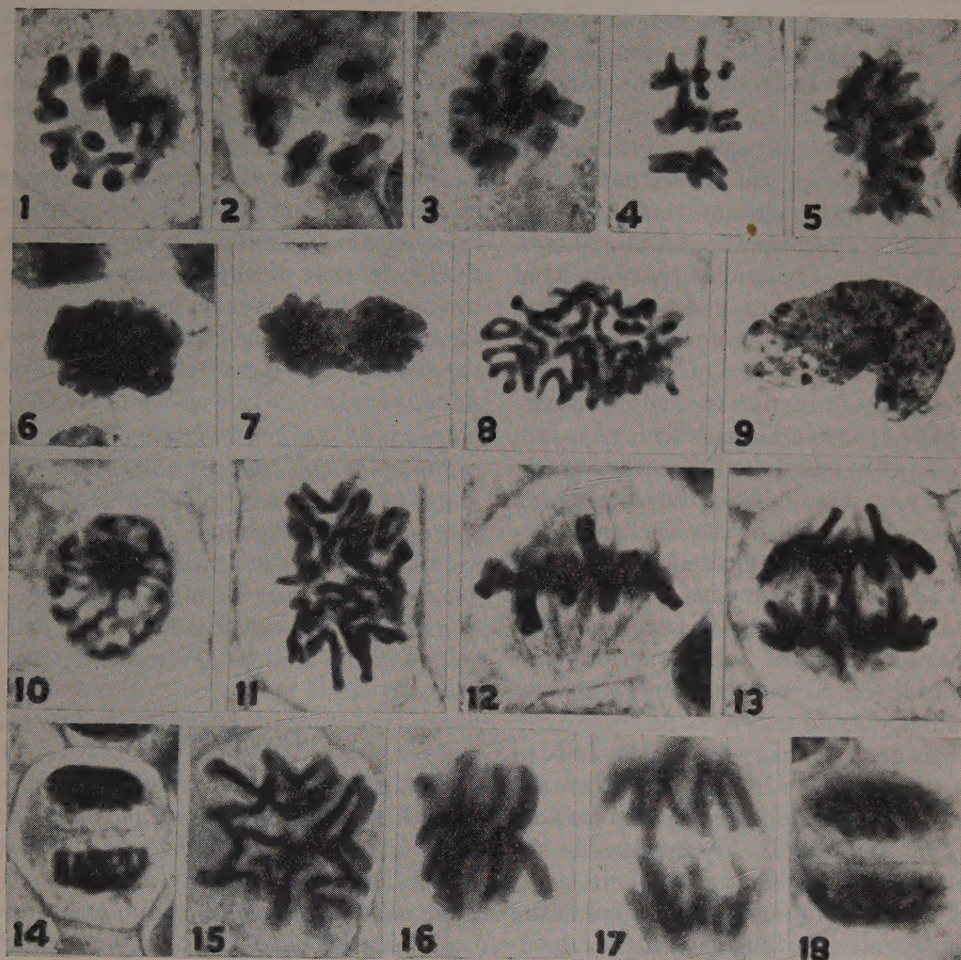
treatments, reached a maximum size at the end of 96 hours. On being returned to tap water, all the treated roots, including those showing tumors, resumed growth. The new growth was noticeably whiter than the darkened older portion.

In a series of preliminary experiments with sulfanilamide at 0.125, 0.25, and 0.5% concentration in tap water at 20°C. , the elongation of roots in the two lower concentrations was inhibited, but tumor formation did not occur. Cells from these roots showed late prophase and metaphase stages with slightly shortened chromosomes and a much slower rate of division than in control roots. At 0.5% concentration sulfanilamide had a more pronounced effect on mitosis. Preliminary examinations of roots at hourly intervals up to 10 hours following the start of treatment showed a progressive shortening of the chromosomes at the late prophase and metaphase stages up to 3 hours, when the maximum contraction appeared to be reached. Because the changes occurred very slowly, later examinations were made at 6, 12, 24, 48, 72, and 96 hours after immersion in the solution.

Mitosis was found to occur in a few cells throughout the 96-hour period of treatment but at such a slow rate and in so few cells that no visible elongation of the treated roots took place during this period. The prophase nuclei responded to sulfanilamide by developing unusually contracted and condensed chromatids. In early prophases the chromosomes were scattered throughout the nucleus. Spiralization of the chromatids could rarely be observed, since it was somewhat obscured by condensed, densely stained material. While still in the mid-prophase stage, the chromosomes were markedly contracted (fig. 1). In later prophases they were scattered around

the inner periphery of the nuclear membrane, the center of the nucleus being clear (fig. 2). In one series of treatments no prophase stages were observable at 48 hours. This occurred under the same experimental conditions as in the other treatments but was not observed again.

The metaphase figures containing the much shortened chromosomes appeared flat when compared with the same stages in the controls (figs. 3, 15). The chromatids were characteristically parallel to each other throughout the metaphase and remained thus until disruption of the



FIGS. 1-18.—Figs. 1-9, cells after treatment with 0.5% sulfanilamide: fig. 1, prophase, 12 hours; fig. 2, late prophase with shortened chromosomes, 12 hours; fig. 3, metaphase with shortened chromosomes, 12 hours; fig. 4, metaphase with disrupted spindle, 48 hours; fig. 5, early reversion stage, metaphase, 96 hours; fig. 6, reversion stage, metaphase, 48 hours; fig. 7, reversion stage, anaphase, 48 hours; fig. 8, metaphase, tetraploid, 96 hours; fig. 9, lobed nucleus from cortical cell of tumor, 96 hours. Figs. 10-14, cells treated with other sulfa- compounds: fig. 10, prophase, sulfathiazole, 12 hours; fig. 11, metaphase plate, sulfadiazine, 6 hours; fig. 12, metaphase, side view, sulfapyridine, 96 hours; fig. 13, anaphase, sulfapyridine, 96 hours; fig. 14, telophase, sulfamerazine, 48 hours. Figs. 15-18, controls, cells untreated: fig. 15, metaphase plate; fig. 16, metaphase, side view; fig. 17, anaphase; fig. 18, telophase.

spindle mechanism occurred. This breakdown first became evident when the metaphase figure was no longer flattened. Concurrently, the chromatids were repelling each other strongly even before the division of the centromeres (fig. 4). The centromeres appeared to divide, however, before the chromosomes reverted to the metabolic condition. Reversion stages were found in material at 48–96 hours (figs. 5, 6). A number of anaphases in which the spindle mechanism had disrupted and in which the chromosomes were reverting to the metabolic state without completion of the mitotic cycle were found at 48 hours (fig. 7). These cells may possibly have been in a late metaphase or anaphase condition at the beginning of treatment. Binucleate cells resulting from the failure of cell-plate formation were also present; probably these cells had been in a late anaphase or telophase stage when treatment was started, and the consequent breakdown of the spindle mechanism resulted in their development. Metaphase and anaphase figures with the tetraploid number of chromosomes were found 96 hours following the application of sulfanilamide (fig. 8), but no other polyploidy was found.

The greatly enlarged cortical cells of the tumors had a large central vacuole, but their nuclei appeared similar to those of the controls. A few nuclei, in the metabolic condition, were much larger and were possibly tetraploid. A few of the greatly enlarged cells showed lobed nuclei resembling those of certain secretory cells or cells of maturing xylem vessels (fig. 9).

After immersion in 0.5% concentration of sulfanilamide for 48 hours, some bulbs of each series were transferred to tap water. No tetraploidy was found in cells from these roots at 24 and 48 hours after being returned to tap water.

Roots treated with the other sulfacompounds—sulfadiazine, sulfaguandine, sulfathiazole, sulfamerazine, and sulfapyridine—all showed the same general responses. Although there was no visible elongation following the application of the compounds, all stages in the mitotic cycle were found at all intervals during treatments, but with the division rate markedly retarded. The prophase and telophase stages appeared similar to those in controls. The metaphase and anaphase figures showed shortened chromosomes, but the degree of contraction was less than that resulting from treatment with 0.5% sulfanilamide, although similar to that of chromosomes in cells treated with the weaker concentrations of sulfanilamide. The principal effect was the marked slowing of the rate of divisions.

Roots of the one bulb that produced tumors in the sulfapyridine solution failed to show the cytological effects resulting from 0.5% sulfanilamide, except for the enlargement of the cortical cells. The mitotic figures were similar to those in roots from the other bulbs treated with sulfapyridine which developed no tumors.

Discussion

The results of this investigation confirmed the previous reports (8, 9) of the gross effects of sulfanilamide in that elongation of the roots ceased and tumors were developed in the morphological region of elongation. In the present experiments tumors were never formed on more than half the roots of any bulb. No visible differences could be detected between the nuclei of roots that formed tumors and those that did not, whether treated or untreated, except that an occasional nucleus in the tumor showed a lobed outline. Tumor formation did not occur following treatment with the other

sulfa- compounds, except in one bulb treated with sulfapyridine. Enlargement of the cortical cells to form tumors was therefore probably a result of a physiological disturbance in these cells.

All the sulfa- compounds prevented visible elongation of roots, while those of the controls grew to the bottom of the containers during the same time interval. Mitosis occurred in some cells of all the treated roots, except for one series of treatments with sulfanilamide in which mitosis was completely blocked at 48 hours. This agrees with the results of PETERS (8), although complete blocking of mitosis was, however, not again observed in any of the four repetitions with sulfanilamide.

The results with 0.5% concentrations of sulfanilamide were generally the same as those found by PETERS except that the responses were slower than he reported. Reversion stages were found at 48-96 hours after treatment began. PETERS reported them as early as 3 hours after treatment, with blocked metaphases in tetraploid nuclei as early as 8 hours. Only after 96 hours of treatment were tetraploid nuclei observed in the present experiments. In some of these the chromosomes appeared in flattened metaphase plates, although others showed apparently normal anaphase configurations during the time in which the roots were still immersed in the sulfanilamide solution.

According to PETERS, the prophase stages in treated roots were similar to those of the controls, with the chromatids a little more distinct. In the writer's material the chromatids appeared somewhat obscured by a slight cloudiness around them during the early prophase stages and were therefore less distinct than in the controls. In later prophase stages, however, the chromatids were more distinct than in the controls. Dur-

ing the late prophase stages the chromosomes may be as much shortened as they become in any of the metaphase stages. These latter stages were similar to those resulting from colchicine treatment, according to figure 4 of BERGER and WITKUS (1) except that this figure showed no nuclear membrane. They interpreted this as representing a disruption of the spindle mechanism at metaphase, with the chromosomes grouped around an achromatic sphere. PETERS reported that, following such a grouping, reversions to an active metabolic condition occasionally occurred. In the present experiments all the chromosomes appeared to reach the metaphase-plate stage before there was any indication of the disruption of the spindle. It seems probable that a breakdown of the nuclear membrane in a late prophase, when the short highly condensed chromosomes are arranged peripherally around the clear center of the nucleus, would result in the appearance described. Thus, what PETERS and BERGER and WITKUS interpreted as a type of disruption of the metaphase may instead be a late prophase in which the nuclear membrane had been dissolved and in which the chromosomes were reverting to an active metabolic condition without first being arranged in a metaphase plate.

The marked differences in results of treatment with sulfanilamide (0.5% concentration) and of those with the five other sulfa- compounds (0.1% concentrations) were possibly correlated with the lower solubility in water of the latter. This conclusion would be in accord with the findings of ÖSTERGREN and LEVAN (7), who used benzene, cyclohexane, and thiophene and various halogen derivatives of these compounds—monobromobenzene, monochlorobenzene, bromocyclohexane, dibromothiophene, etc.

No explanation can at present be offered for the more rapid results obtained by PETERS with 0.5% solutions of sulfanilamide than the consistently slower effects observed during the course of this investigation.

For demonstrating mitosis to the beginning student it is suggested that onion roots be immersed for 6 hours, before being fixed, in a 0.1% concentration of one of the sulfa- compounds. Under these conditions all stages of the mitotic cycle are essentially normal except that the chromosomes are thicker and shorter and therefore less entangled. Flat metaphase figures are obtained in which the sixteen chromosomes are clearly visible and in which the chromatids also show more clearly in the late prophase than in untreated material.

Summary

1. Onion roots approximately 1 inch long were immersed in solutions of 0.5% sulfanilamide and of 0.1% in tap water of the following sulfa- compounds: sulfadiazine, sulfaguanidine, sulfamerazine, sulfapyridine, and sulfathiazole. These solutions were at or slightly above the maximum solubility of these compounds in water at 20° C. The solutions were changed every 24 hours, as were the controls in tap water. Fixations of the roots were made after 6, 12, 24, 48, 72, and 96 hours of treatment. Some bulbs from each series were returned to tap water after 48 hours and others after 96 hours.

2. There was immediate cessation of visible root elongation in all the treatments, but approximately half the roots of the bulbs treated with sulfanilamide developed tumors within 48 hours, owing to enlargement of the cortical cells in the region of elongation. In only one other treatment were such tumors formed—on about half the roots of one bulb immersed in sulfapyridine.

3. In roots treated with sulfanilamide normal-appearing mitosis continued in a small number of the cells of the cortex and apical meristem throughout the 96-hours period of treatment. The duration of the mitotic process seemed to be materially lengthened. Disruption of the spindle mechanism and delay in the separation of the centromeres of the chromosomes were observed. As a result of the inhibition of the spindle mechanism, reversion stages occurred which resulted in the return of the anaphase chromosomes to the metabolic condition without completion of the mitotic cycle and in the production of tetraploid metaphase and anaphase figures within 96 hours after application of sulfanilamide. In no case, however, was a tetraploid cell found in division after normal growth had been resumed in tap water. Binucleate cells, resulting from the failure of the formation of the cell plate, were observed after 48 hours of treatment. The nuclei of the cortical cells in the enlarged regions appeared normal in most cells, although some nuclei were much larger than others and than those of the controls. These large nuclei were probably tetraploid. A few other nuclei showed irregular, lobed outlines. No micronuclei were found, in contrast to the report of PETERS. The only polyploidy observed was tetraploidy.

4. Mitosis was completely blocked at 48 hours in one series of treatments with sulfanilamide. In these roots no mitotic activity and no characteristic tumor formation occurred.

5. The other five sulfa- compounds induced essentially the same responses in all the roots. Although there was no visible elongation, mitosis nevertheless continued at a slow rate throughout the 96 hours of treatment. Even though the chromosomes were shorter than in the controls, the degree of contraction was

not so great as had occurred in cells treated with sulfanilamide. On return to tap water all treated roots resumed normal growth.

6. The markedly different responses to sulfanilamide and to the other sulfa-compounds occur possibly because of the greater solubility in water of sulfanilamide than of the others.

The writer wishes to express appreciation to Dr. J. M. BEAL and to other members of the Department of Botany at the University of Chicago for their advice and assistance during the course of this investigation.

DEPARTMENT OF BOTANY
RHODE ISLAND STATE COLLEGE
KINGSTON, RHODE ISLAND

LITERATURE CITED

1. BERGER, C. A., and WITKUS, E. R. A cytological study of c-mitosis in the polysomatic plant *Spinacia oleracea* with comparative observations on *Allium cepa*. Bull. Torr. Bot. Club 70:457-466. 1943.
2. BLAKESLEE, A. F. Dédoublément du nombre de chromosomes chez les plantes par traitement chimique. Compt. Rend. Acad. Sci. (Paris) 205:476-479. 1938.
3. ———. Doubling chromosomes by means of colchicine. Gard. Chron. III, 103:270-271. 1938.
4. EIGSTI, O. J. A cytological study of colchicine effects in the induction of polyploidy in plants. Proc. Natl. Acad. Sci. 24(2):56-62. 1938.
5. ———. A comparative study of the effects of sulfanilamide and colchicine upon mitosis of the generative cell in the pollen tube of *Tradescantia occidentalis* (Britton) Smyth. Genetics 27:141-142. 1942.
6. JENSEN, HOLGAR. Modern breeding technique for trees and shrubs. K. Lantbruks-Akad. Handl. och Tidskr. (Stockholm) 82:330-340. 1943.
7. OSTERGREN, G., and LEVAN, A. The connection between c-mitotic activity and water solubility in some monocyclic compounds. Hereditas 29:496-498. 1943.
8. PETERS, J. J. Cytological effects of sulfanilamide on *Allium cepa*. BOT. GAZ. 107:390-392. 1946.
9. TRAUB, H. P. Effect of sulfanilamide and other sulfa-compounds on nuclear conditions in plants. Jour. Hered. 32:157-159. 1941.

